REMARKS

In response to the election/restriction requirement of January 9, 2003, the Applicant elects with traverse Group VIII and submits herewith remarks which demonstrate that the inventions listed in Groups I-IX and XI-XII relate to a single general inventive concept, namely, the synthesis of a high value pharmaceutical protein in a transgenic plant via plastid expression for pharmaceutical protein production.

The Applicant acknowledges the Examiner's comments concerning Blowers et al. that this invention is not "special" because it does not constitute an advance the prior art. Applicant respectfully submits that Blowers et al. (WO 99/05265) is not enabled and therefore fails to teach plastid transformation vectors comprising a plastid promoter, a selectable marker sequence, a nucleic acid sequence encoding a protein, 5' and 3' UTRs, and flanking sequences that are homologous to plastid DNA. Applicant submits that Blowers et al. only teaches vectors designed to confer herbicide resistance.

The concept of the current invention is totally different from that of Blower et al. While Blowers et al. attempt to confer a trait beneficial to a plant (herbicide resistance), the current invention confers no advantage to the plant. In contrast, the current invention uses chloroplasts as bioreactors to make pharmaceutical proteins, which would be of value to humans, rather than of value to plants as is taught in Blowers et al. The current Application's unique discovery mandates advancement of several novel concepts. For example, expression of human blood proteins in chloroplasts require not only synthesis but also requires chaperones to fold them properly and form disulfide bonds to stabilize folded structures. Until the current invention, assembly of multi-subunits and disulfide bond formation had never been demonstrated for a foreign protein. For example, conclusive proof for the assembly of pentameric structure of cholera-toxin B subunit as a vaccine antigen capable of binding to GM1 intestinal epithelial surface protein has been demonstrated for the first time in transgenic chloroplasts. Similarly, Human Serum Albmin requires 17 disulfide bonds.

Second, the protein produced in chloroplasts should be taken out of transgenic plants. This necessitates the need to develop novel purification strategies. Such strategies using biopolymers that are capable of precipitating proteins using different temperature cycles (avoiding the use of expensive column chromatography) was developed in the current invention.

Third, the foreign proteins should be produced in large quantities. Blowers et al however,.illustrate only small quantities of enzymes, because they function in a catalytic manner. The Applicant has demonstrated that the insertion of 5' and 3' UTRs and codon optimization enhance translation of foreign genes. In complete contrast, herbicide resistance genes of Blowers et al., were not of human origin and did not require codon optimization. Finally, for oral delivery of therapeutic proteins from plant cells, several novel experimental strategies were developed. None of these unique requirements, which is shared by all of the claims in Groups I-IX and XI-XII were shown, suggested, or demonstrated by Blowers et al.

Moreover, as is illustrated in the attached declaration of Henry Daniell, the vector constructs taught by Blowers et al. are non-functional, and thus unenabled.(see attached declaration).

The Applicant however has shown, through a multitude of examples vectors designed to express **pharmaceutical proteins**. As an illustrative Example of the vector designs capable for expressing a pharmaceutical protein, the Applicant has created plastid transformation vectors to express Human Serum Albumin, Insulin, and Interferon. Consequently, the expression of a pharmaceutical protein via the plastid is the common technical and special feature shared by Groups I-IX and XI-XII. Applicants respectfully submit that Blowers et al does not show this common technical feature. Rather and in spite of its lack of functionality, Blowers et al., merely discusses conferring herbicide resistance to a plant cell. The Examiner's attention is invited to page 9, lines 4-11 of Blowers et al.

> Consistent with the foregoing, the present invention provides methods of transforming the plastids (particularly proplastid) genome of a plant with a nucleic acid comprising one or a plurality of selectable marker genes that confer herbicide resistance to the transform plant cells. More particularly, such selectable marker genes express an enzyme that inactivates an herbicide. Gene expression in plastids/proplastids transformed according to the invention occurs at levels that enable a plant having the transformed plastids/proplastids to survive contact with at least the minimal amount of herbicide that would kill an otherwise similar wild-type plant.

In view of the foregoing, the Examiner's attention is further invited to page 6, third paragraph and page 51, summary of invention of the Applicant's specification:

This invention synthesizes high value pharmaceutical proteins in transgenic plants by chloroplast expression for pharmaceutical protein production. Chloroplasts are suitable for this purpose because of their ability to process eukaryotic proteins, including folding and formation of disulfide bridges, thereby eliminating the need for expensive post-purification processing. Tobacco is an ideal choice for this purpose because of its large biomass, ease of scale-up (million seeds per plant) and genetic manipulation. We use poly(GVGVP) as a fusion protein to enable hyper-expression of insulin and accomplish rapid one step purification of fusion peptides utilizing the inverse temperature transition properties of this polymer. We also use insulin-CTB fusion protein in chloroplasts of nicotine free edible tobacco (LAMB 605) for oral delivery to NOD mice.

(Cholera Toxin Subunit B filing) This invention includes expression of native cholera toxin B subunit gene as oligomers in transgenic tobacco chloroplasts which may be utilized in connection with large-scale production of purified CTB, as well as an edible vaccine if expressed in an edible plant or as a transmucosal carrier of peptides to which it is fused to either enhance mucosal immunity or to induce oral tolerance of the products of these peptides.

The present invention develops recombinant DNA vectors for enhanced expression of human serum albumin, insulin-like growth factor I, and interferon-$\alpha 2$ and 5, **via chloroplast genomes of tobacco, optimizes processing and purification of pharmaceutical proteins** using chloroplast vectors in *E. coli*, and obtains transgenic tobacco plants. The transgenic expression of proteins or fusion proteins is characterized using molecular and biochemical methods in chloroplasts. Existing or modified methods of purification are employed on transgenic leaves. Mendelian or maternal inheritance of transgenic plants is analayzed. Large scale purification of therapeutic proteins from transgenic tobacco and comparison of current purification methods in *E. coli* or yeast is performed, and natural refolding in chloroplasts is compared with existing *in vitro* processing mehtods; Comparison/characterization (yield and purity) of therapeutic proteins produced in yeast or *E. coli* with transgenic tobacco chloroplasts is performed, as are *In vitro* and *in vivo* (pre-clinical trials) studies of protein biofunctionality.

The aforementioned illustrates that the Applicant has disclosed vectors capable for the production of high value pharmaceutical proteins by utilizing fusion proteins to hyper-expression

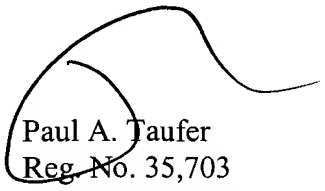
and purify such pharmaceutical proteins as insulin, HSA, and interferon-α2 and 5. Prior to the Applicant's discovery it was not known whether disulfide bonds in plant chloroplasts existed. Consequently, it was not known whether it was possible to express the aforementioned pharmaceutical proteins in chloroplasts.

Nowhere does the Blowers et al. disclosure, teach or suggest the inventive concept of creating a fusion protein which allows hyper-expression and one step purification process for pharmaceutical proteins. Furthermore, Blowers et al. provides no evidence as to the formation of disulfide bonds in plant chloroplasts. Hence, Blowers et al. is not enabling and not effective prior art.

The Applicant respectfully submits that Groups I-XII share a single inventive concept and as a result should be examined as one group. Applicant's acknowledge the Examiner's observation concerning Group X as it relates to a process for recovering a biopolymer. As a result, Applicant's request that Groups I-IX and XI-XII be examined as a single group, and Group X be removed from consideration in the current examination.

In accordance with the Examiner's helpful suggestion, the Applicant has amended the specification to incorporate proper sequence identifiers. Further, the Applicant submits herewith an updated copy of the Sequence Listing and is in full compliance of the sequence rules. In view of the foregoing, Applicant's respectfully request examination of Groups I-IX and XI-XII.

Respectfully submitted,



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